Message

From: Nadine Kotlarz [nkotlar@ncsu.edu]
Sent: 3/18/2019 2:33:59 PM
To: Collier, David [COLLIERD@ecu.edu]
CC: Claire Critchley [cecritch@ncsu.edu]; Adrien Wilkie [aawilkie@ncsu.edu]; Jane Hoppin [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=userebcfc262]; Strynar, Mark [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=5a9910d5b38e471497bd875fd329a20a-Strynar, Mark]
Subject: Re: new disease and cure

Hi David,

Here are PIDs for the urine samples to send to EPA. These should all have at least 75 mL urine left. Please give us a heads up when you're ready to ship them so we can make sure Mark and I will be at EPA to receive them. Claire and I will work on getting the labels and tubes ready for you to pick up on Wednesday.

Thank you,
Nadine

High Nafion byproduct 2:

PID	SAMPLE_DATE
772	NOV_2017
359	NOV_2017
347	NOV_2017
385	NOV_2017
719	NOV_2017
697	NOV_2017
496	NOV_2017
792	NOV_2017
702	NOV_2017
363	NOV_2017
423	NOV_2017
686	NOV_2017
344	NOV_2017
677	NOV_2017
454	NOV_2017
326	NOV_2017
349	MAY_2018
764	MAY_2018
412	NOV_2017
393	NOV_2017

Randomly selected from the rest:

PID	SAMPLE_DATE
785	NOV_2017
784	MAY_2018
766	NOV_2017
765	NOV_2017
747	NOV_2017

733 NOV_2017
708 NOV_2017
683 NOV_2017
516 MAY_2018
509 MAY_2018
498 NOV_2017
474 NOV_2017
464 NOV_2017
458 NOV_2017
450 NOV_2017
427 NOV_2017
416 NOV_2017
414 NOV_2017
394 NOV_2017
380 NOV_2017

On Fri, Mar 15, 2019 at 5:14 PM Collier, David <COLLIERD@ecu.edu> wrote:

Hi Nadine,

The updated spread sheet attached includes urines from May sampling in Wilmington with volume estimates included.

I understand that you will need some empty Greiner tubes for contaminant testing. I'll bring as many as you need when I come to NCSU on Wed 3/20.

I also expect that I'll pick up labels for the 40 urines that you identify (20 high nafion and 20 random) for immediate sub-aliquoting as well as at least 120 of the 15 ml Falcon tunes (3 each for EPA samples) and at least 40 of the 50 ml Falcon tubes (1 each for extra urine).

While the urines are thawed I would like to also add two x 1 ml aliquots in Nunc tubes – possibly to be used to metabolmics.

Hence will need following set of labels:

Volume urine	tube type	label	purpose
1 ml	Nunc	PID**USS1**	**urine** small subsample – metabolmics or other
1 ml	Nunc	PID**USS2**	**urine** small subsample – metabolmics or other

5 ml	Greiner	PIDULT1	urine long term storage
5 ml	Greiner	PIDULT2	urine long term storage
10 ml	15 ml Falcon	PIDUEPA1	urine EPA analysis
10 ml	15 ml Falcon	PIDUEPA2	urine EPA analysis
10 ml	15 ml Falcon	PIDUEPA3	urine EPA analysis
<50 ml	50 ml Falcon	PIDUXTRA	urine extra for storage

It would be easiest for me (I think) if labels are made and NOT applied to tubes – best done by me when aliquoting.

Sincerely,

David

From: Nadine Kotlarz [mailto:nkotlar@ncsu.edu]
Sent: Wednesday, March 13, 2019 9:44 AM
To: Collier, David <COLLIERD@ecu.edu>
Subject: Re: new disease and cure

Thanks David

It probably would be helpful to weigh the May urine samples eventually so that we know how much we have... but if it's pretty easy to estimate ~75 mL visually, then an inventory with a "yes" or "no" for each PID sounds fine for now.

When I have your inventory for the May sampling I'll merge your weight information with my Nafion byproduct 2 numbers for all Wilmington PIDs and then I'll find 20 high and 20 random PIDs that have enough.

Nadine

On Tue, Mar 12, 2019 at 6:09 PM Collier, David <COLLIERD@ecu.edu> wrote:

Nadine – here are the volumes for the randomly chosen urines. As you can see only about 11 of these have sufficient volume.

DC

PID	ml urine
307	65
323	54
329	23
344	89
369	105
382	44
389	11
398	64
408	32
415	38
422	82
450	84
451	57
490	108
517	30
531	100
705	98
706	17
748	126
751	8
752	79
753	110
758	8
777	77
787	26

From: Nadine Kotlarz [mailto:nkotlar@ncsu.edu]
Sent: Tuesday, March 12, 2019 3:34 PM
To: Collier, David <COLLIERD@ecu.edu>
Cc: Jane Hoppin <jahoppin@ncsu.edu>; Detlef Knappe <knappe@ncsu.edu>; Claire Critchley <cecritch@ncsu.edu>; Adrien Wilkie <aawilkie@ncsu.edu>
Subject: Re: new disease and cure

Hi David,

Thanks for your patience.

We hammered out a plan for the urine today. Let's start with sending to EPA urine for 40 Wilmington participants: 20 people with high Nafion byproduct 2 levels and 20 people randomly selected from the rest.

PIDs for highest Nafion byproduct 2 blood levels:

1. 779
2. 772
3. 348
4. 321
5. 403
6. 392
7. 300
8. 359
9. 347
10. 385
11. 337
12. 325
13. 719
14. 497
15. 697
16. 496
17. 792

18. 702
19. 335
20. 363
21. 492
22. 506
23. 460
24. 739
25. 695

PIDs for randomly selected participants:

1. 753
2. 751
3. 450
4. 415
5. 531
6. 705
7. 408
8. 758
9. 344
10. 517
11. 777
12. 398
13. 752
14. 490
15. 748
16. 323

17. 451

18. 706

19. 422

20. 382

21. 787

22. 389

23. 369

24. 329

25. 307

For each PID above, can you

1. Check if there is sufficient urine (> 75 mL). Right now, we don't want to work with samples that have limited volume. If there's plenty of urine, proceed with step 2. If not, move on to the next PID in the list. I provided 25 per category above so you can skip a few. We'd want at least 20 per category sent to EPA.

2. Create 2 x 5 mL aliquots in Greiner tubes

3. Create 3 x 10 mL aliquots in 15 mL falcon tubes. Label the 15 mL falcon tubes:

PIDUEPA1

PIDUEPA2

PIDUEPA3

Send the 15 mL tubes to EPA.

4. Create 1 x 50 mL aliquot in 50 mL falcon tube. Label the 50 mL falcon tube:

PIDUXTRA

Please keep the 50 mL tube at ECU for now.

5. Keep the remaining urine in the cup at -80C at ECU. If there's nothing left, throw the cup away.

Please do not fill the falcon tubes to the top. When they freeze, the urine will expand and could break the tube. Please let Claire know which PIDs you'll be sending. Then we can give you the falcon tubes and labels when you're at state on Mar 20.

Does this plan sound ok?

Nadine

On Mon, Mar 11, 2019 at 1:12 PM Collier, David <COLLIERD@ecu.edu> wrote:

> Nadine:
>
> The "big thaw" does refer to urine in sample cups but only to urines collected in Wilmington in November 2017 and May 2018.
>
> For samples collected in Fayetteville Feb 2019 we were much more proactive and prepared two sub-aliquots of 5 ml each in Greiner tubes called "XXXULT1" and "XXXULT2" where "ULT" stands for urine long-term storage. These ULT1 and ULT2 samples are well organized in freezer boxes with an inventory map and I can easily pull any particular samples you need from Fayetteville. There are a handful of exceptions where there was insufficient urine to prepare sub-aliquots and all residual urine remains frozen in the specimen cup. We of course prepared 1 ml samples in Nunc tubes ("XXXUVML") that have already gone to Vidant Medical Labs for clinical analysis. The balance of the urine sample beyond the 2 x 5 ml sub-aliquots remains in the original cup. Volumes of residual urine range from 10 ml to more than 100 ml. (I can send you the list of urines in order collected and the residual volumes as estimated by weighing). All of these residual urines in cups went to NCSU with Jane frozen on dry ice and unless someone has organized them they are just in a jumble in boxes in a freezer there.
>
> Back to the Wilmington samples: The frozen cups are in a jumble in boxes – however in preparation for thawing and sub-aliquoting I envision getting them into some semblance of order and so could find the 20 samples you are most interested in starting with and sub-aliquot them first. This is appealing to me b/c it does not require doing the entire sample set – a daunting task – before you can get access to some samples, and your work will probably inform the size and number of aliquots that might be best for the rest of the samples (i.e. I wait for the big thaw until you have a better idea about volumes needed based on your detection limits).

Urine volume in the cups ranges from 10 ml to more than 100 ml. Hence for some subjects we would need to prepare twenty x 5 ml aliquots to empty the cups – and this would not be a space saver. For some subjects all the urine would fit into 2 – 4 Greiner tubes and we could throw away the empty cup – which would be a space saver....

At this point I was thinking of preparing at least one 1 ml aliquot in a Nunc tube to save for metabolomics and two 5 ml aliquots in Greiner tubes. I am inclined to want to prepare more tubes now than go back later – both for ease as well as sparing the samples from another freeze thaw cycle.

Thoughts?

David

From: Nadine Kotlarz [mailto:nkotlar@ncsu.edu]
Sent: Monday, March 11, 2019 11:57 AM
To: Collier, David <COLLIERD@ecu.edu>
Subject: Re: new disease and cure

Hi David,

I have a few clarifying questions so I can get a better sense of what's going on.

The "thaw" refers to the urine in the cups only, is that right?

About how much urine is left in the cups (for Wilmington and Fayetteville)?

Do we want to aliquot all of the urine left in the cups to save on storage space?

If we wanted to start PFAS analysis on urine from the 20 participants that had the highest Nafion byproduct 2 levels, could you find 20 specific urine samples easily without having to do a big thaw on all samples?

Nadine

On Mon, Mar 11, 2019 at 11:41 AM Collier, David <COLLIERD@ecu.edu> wrote:

great!

DC

David N. Collier, MD, PhD, FAAP

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From: Nadine Kotlarz <nkotlar@ncsu.edu>
Sent: Monday, March 11, 2019 10:41 AM
To: Collier, David
Subject: Re: new disease and cure

David,

I'm speaking with Mark about the aliquoting of urine samples for PFAS analysis. I'll get back to you soon.

Nadine

On Thu, Mar 7, 2019 at 10:22 PM Adrien Wilkie <aawilkie@ncsu.edu> wrote:

Hi David and Jane,

I can email Rob first thing tomorrow AM to inquire about the label maker.

A couple of logistical questions:

- So all of the PID urine samples (Wilmington Nov and May; Fayetteville Feb) will need 4 new labels printed? Or was the Fayetteville urine prepped for storage how we want the Wilmington samples to be stored?
- Is the scan bar on the original labels still required for the new labels or can we have little stickers with the needed info (e.g. ULT1###, ULT2###, ULT3###, ULT4)?

Thanks,

Adrien

Adrien Wilkie, MSPH

Research Assistant

GenX Exposure Study

On Thu, Mar 7, 2019 at 2:54 PM Collier, David <COLLIERD@ecu.edu> wrote:

> Bless you! Jane you are becoming MY favorite collaborator.
>
> I am thinking that we could sub aliquot two 1 ml and two 7 ml aliquots (total of 4) while thawed. If others think we should do more aliquots now is the time to speak!
>
> DC
>
> **From:** Jane Hoppin [mailto:jahoppin@ncsu.edu]
> **Sent:** Thursday, March 07, 2019 2:36 PM
> **To:** Collier, David <COLLIERD@ecu.edu>
> **Cc:** Claire Critchley <cecritch@ncsu.edu>; Adrien Wilkie <aawilkie@ncsu.edu>; Nadine Kotlarz <nkotlar@ncsu.edu>
> **Subject:** Re: new disease and cure
>
> Yes, we can do this.

Can someone talk to Rob about his new label maker and see if we can use it to make these? Otherwise we'll print some more the other way.

On Thu, Mar 7, 2019 at 1:30 PM Collier, David <COLLIERD@ecu.edu> wrote:

I have begun sorting/organizing labels from Wilmington. After several hours I developed a serious case of "labelphobia". Signs and symptoms of labelphobia include long hours spent shuffling small unruly pieces of paper that don't lay flat and catch the wind, buildup of multiple file folders and envelopes labelled in a vain attempt to organize said fragments, and a sense of impending dread that despite one's best measures many labels may be missing. I have heard that special ordering new labels provides immediate relief from labelphobia…..

So in all seriousness I think ordering a limited set of new labels dedicated to labeling sub aliquots of urine would be a GREAT use resources. It would save time sorting labels now, would really save time in the actual sub-aliquoting of the urines, would facilitate book-keeping and likely will reduce errors and confusion in the future that might be associated with hodge-podge use of left over labels.

Please consider helping a dying man!

D

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